Growth of Pteridium aquilinum (L.) Kuhn Gametophytes in Submerged Culture

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The culture of fern gametophytes under aseptic conditions is commonly carried out on various mineral solutions with or without agar. In these methods the ferns develop on the surface of the culture medium. In order to obtain a higher yield of gametophyte tissue, an attempt was made to develop a method for growing these plants in submerged, agitated, liquid cultures, thereby utilizing the entire volume of the medium instead of the surface alone. Since submerged gametophytes develop very slowly, the plants were aerated by bubbling sterile air through the medium. This paper will describe the method developed and will compare the development of gametophtyes grown by this method to that of ferns grown by more standard culture procedures.

Two papers were found in the literature that described the development of lower archegoniates in agitated cultures: Nakazawa (1956) reported on the germination of *Equisetum* spores in aerated submerged cultures, and Machlis and Doyle (1962) described a technique for the growth of liverworts, mosses, and *Equisetum* in flasks agitated on a rotary shaker. Voeller (1964) has described the growth of fern gametophytes in submerged cultures that were not agitated; the development was stimulated by the addition of coconut milk to the medium.

Spores of the Bracken Fern, *Pteridium aquilinum* (L.) Kuhn, were collected in September, 1963, in Pulaski County, Indiana, and stored under refrigeration. The spores were filtered through six layers of lens paper to remove sporangia. For agitated cultures or cultures using a medium solidified with agar,

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the spore surface was sterilized with Chlorox (20%, for 15 sec) and suspended in 0.2% "Tween 20." Dry, unsterilized spores were used when they were to be floated on the surface of the liquid medium. In all cases the medium was composed of Moore's salt solution plus Nitsch's modified trace element solution (Kelley and Postlethwait, 1962). All cultures were maintained at 24° C and were illuminated continuously with fluorescent lamps (approximately 200 ft-c.).

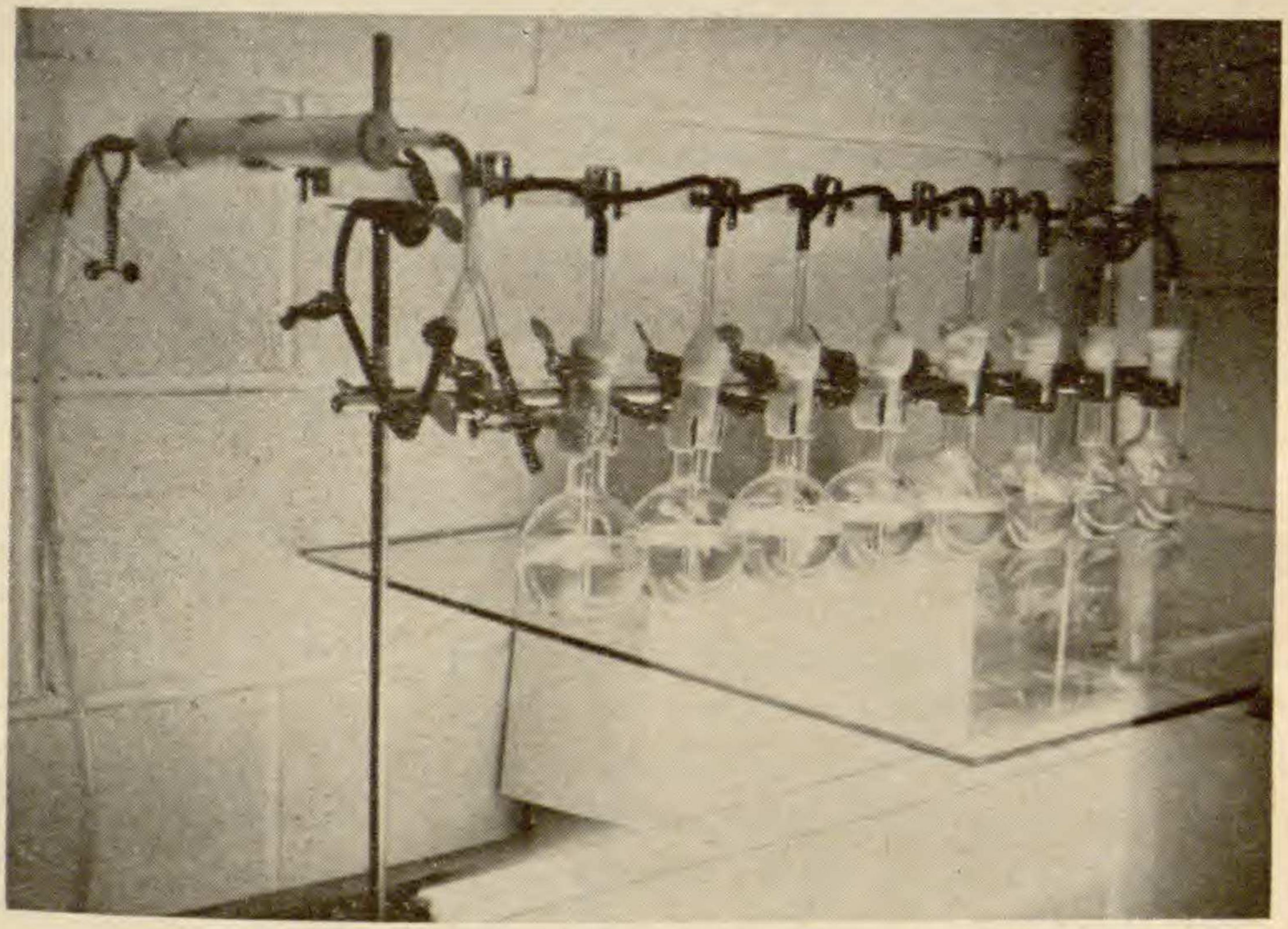
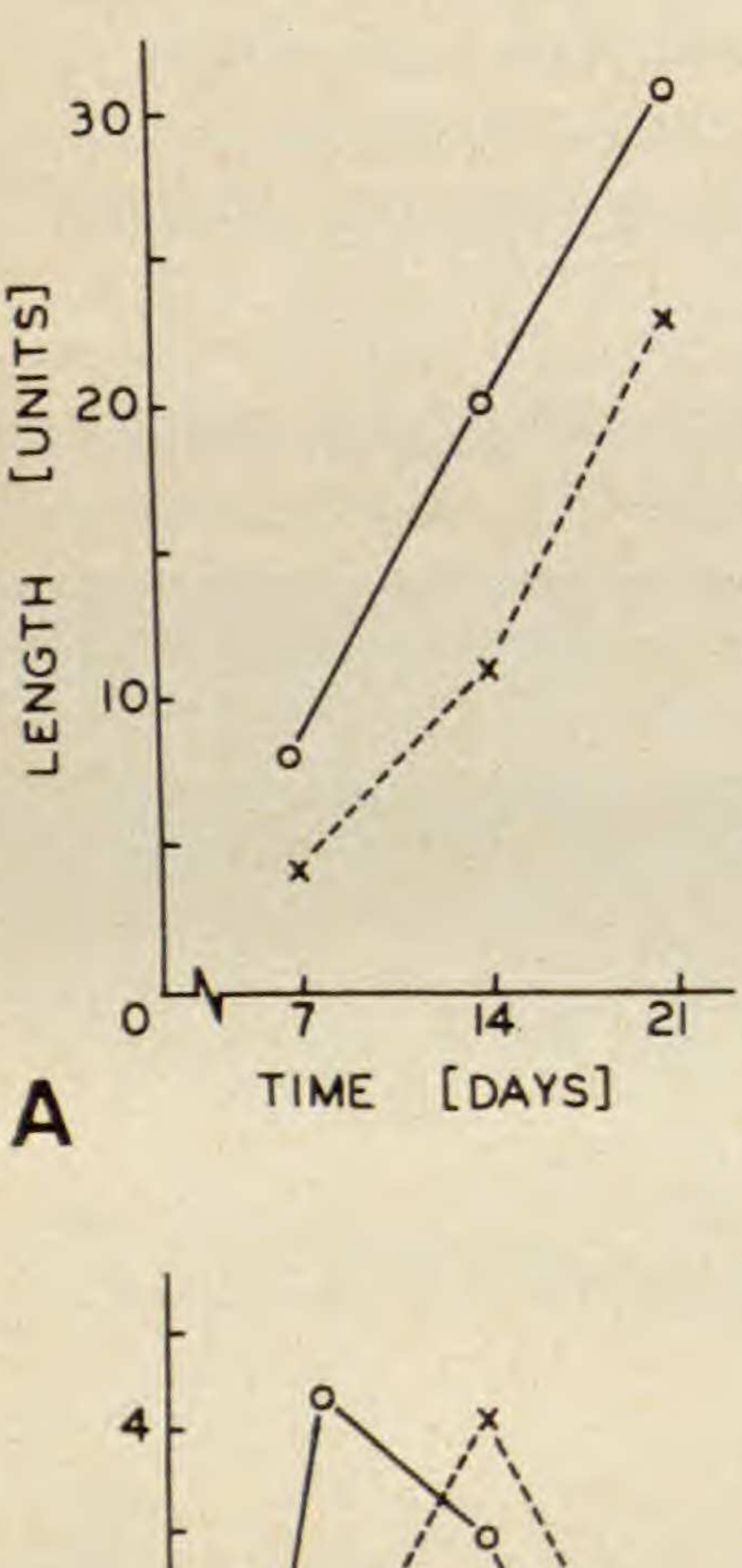


FIG. 1. ARRANGEMENT OF CULTURE APPARATUS.

The general arrangement of the apparatus is shown in *Fig. 1*. Only half of the apparatus was photographed; the rest was a mirror image directly in front of the section shown (removed for clarification). Air was passed through 6 inches of glass wool (tube to upper left of *Fig. 1*) and then through cotton plugs in the air delivery tubes. No attempt was made to measure the rate of air flow.

In the first series of experiments, the gametophytes from agitated cultures were compared to ferns grown in 2-inch Petri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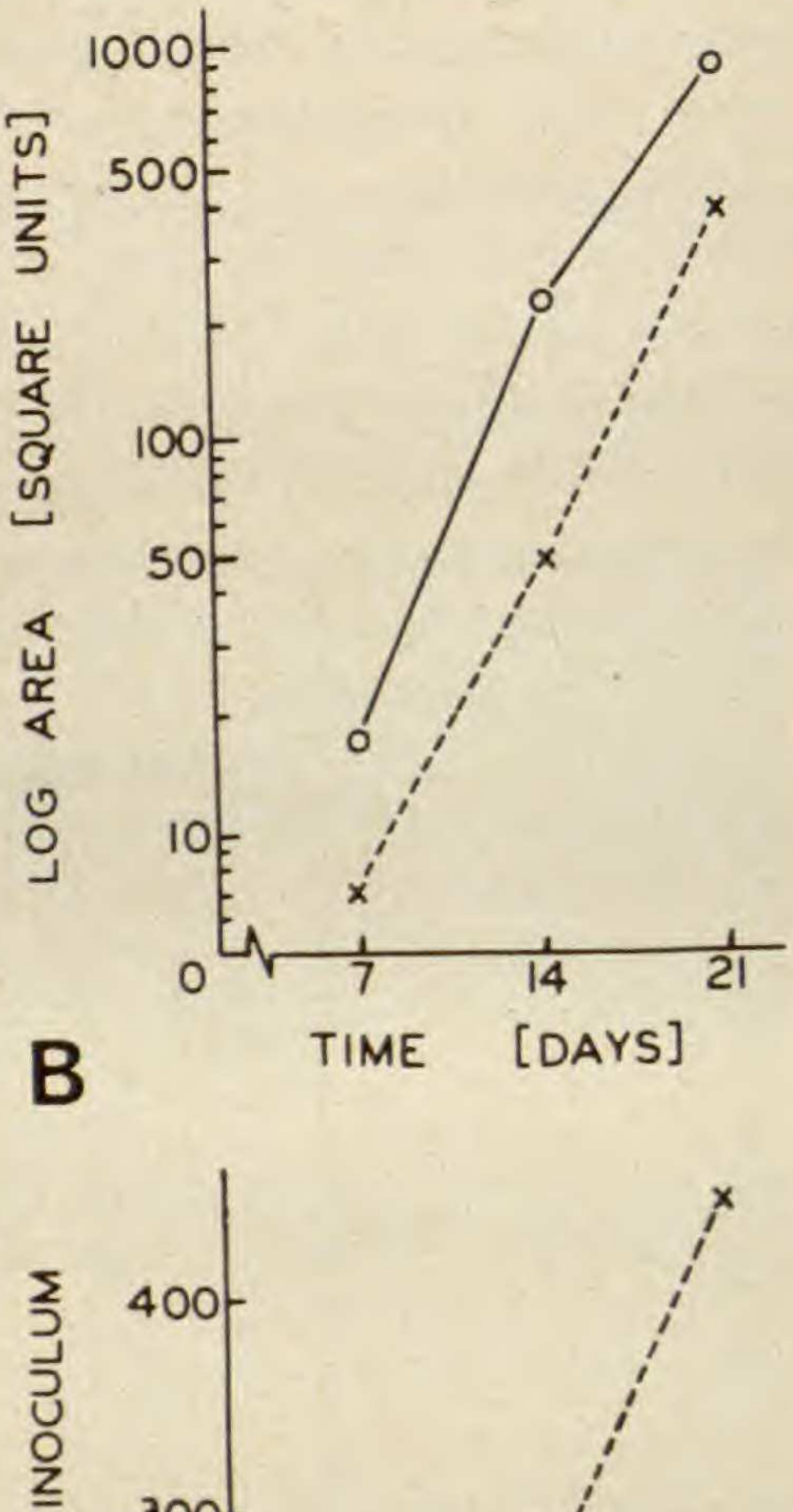
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TIME [DAYS]

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FRESH WT. / SPORE INOCULUM
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dishes containing 10 ml of the medium solidified with 1% agar. Four cultures were examined for each method on the 7th, 14th, and 21st day after inoculation; 50 plants per culture were measured for maximum length and breadth with a microscope equipped with an ocular micrometer. In a second series, the yield was compared for gametophytes grown in agitated culture and in still liquid culture. Statistical differences were determined by the *t*-test (Steel and Torrie, 1960).

Although the aeration resulted in the gametophytes being periodically subjected to a turbulence, the morphogenesis of these plants did not seem to be altered. The plants developed their normal cordate form. Numerous antheridia could be seen on most of the ferns by the 21st day; archegonia were observed within 28 days. When the gametophytes were transferred to still liquid cultures, young sporophytes were formed.

The growth of gametophytes from agitated cultures and from still cultures (medium solidified with 1% agar) was measured by estimation of maximum length and breadth. The mean length (*Pl. 13A*) and area (*Pl. 13B*) on each date were significantly higher for gametophytes from agitated cultures (differences significant at 0.01 level). However, the rate of growth, as indicated by the slope of the curves, was the same in both culture methods. The development of the gametophytes was also measured by the "morphogenetic index" of maximum length divided by maximum breadth (L/B; cf. Mohr, 1956). As the ferns developed in the filamentous manner, the morphogenetic index increased (i.e., the gametophytes grew in length but not in breadth); once two-dimensional growth was initiated, the morphogenetic index became stationary or actually decreased

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PL. 13. COMPARISON OF DEVELOPMENT OF GAMETOPHYTES FROM AGITATED CULTURES (————————) TO THOSE GROWN ON STILL CULTURES (- - - - - - -) ON MEDIUM SOLIDIFIED WITH AGAR (A–C) OR ON LIQUID MEDIUM (D). A. INCREASE IN MAXIMUM LENGTH. B. INCREASE IN AREA (MAXIMUM LENGTH TIMES MAXIMUM BREADTH). C. CHANGE IN MORPHOGENETIC INDEX (L/B: MAXIMUM LENGTH DIVIDED BY MAXIMUM BREADTH). D. FRESH WEIGHT ACCUMULATION. [1 UNIT = 32 μ.]

(i.e., the gametophytes became wider than long). The morphogenetic index of the gametophytes from agitated cultures differed statistically (0.01 level) on the 7th day, but not on the subsequent days (*Pl. 13C*). Therefore it may be concluded that the plants from agitated cultures were more precocious than those on agar-solidified medium, but that the rate of growth (as measured by the rate of increase in length or in area) and the development (as measured by the morphogenetic index) are the same.

Measurement of fresh weight can be used as an index of growth, and is indeed more basic to the goal of this study. Since it is difficult to separate the gametophytes from agar, yield measurements were made by comparing ferns from agitated cultures to those from still liquid cultures. *Plate 13D* shows the fresh weight accumulation to be higher (different at the 0.01 level) in still liquid cultures than in agitated cultures. The rates of growth were also different in the two culture methods.

The submerged culture technique has the same advantages for the study of higher plants as it does for microbes. However, it is also important to determine whether the technique alters the development of the material being studied. For example, Albaum (1938) described "irregular changes in form [which] take place unless the experimental plants are always kept in the same position relative to the light source." In this experiment the gametophytes in agitated cultures were periodically subjected to turbulent movement caused by the air bubbling through the medium. When these gametophytes were compared to those grown on agar-solidified medium, it was found that the rates of growth (increase in length and in area) and the development (changes in morphogenetic index) were the same. The development of *Pteridium aquilinum* gametophytes was not altered by the culture technique.

The major advantage of submerged culture technique is the potential for increased yield of tissue. In this study it was found that the fresh weight accumulation in still liquid cultures

was greater than in agitated cultures. One must therefore conclude that the agitation culture technique in this case is superior to culture on an agar-solidified medium, but is inferior to standard techniques in which the gametophytes develop on the surface of a liquid medium.

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Chromosome Studies in the Polypodiaceae

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The Polypodiaceae are typically epiphytic, rarely terrestrial, mostly tropical ferns. The type genus has been considered one of the largest fern genera by many pteridologists, but Copeland (1947) has construed it narrowly, and includes only about 75 species, mostly in the American tropics. Chromosome counts of about 35 species mostly from southeast Asia have been published (Chiarugi, 1960, Fabbri, 1963, Evans, 1963a), while the American species have been reported by Manton (1951), Knobloch (1962), Evans (1963a, b), Lloyd (1963), Taylor and Lang (1963), and Wagner (1963).